*Institute Report No. 354*

# Acute Oral Toxicity of Nitrosoguanidine in ICR Mice

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*and*
*Don W. Korte, Jr., PhD, LTC, MSC*



MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

September 1989

Toxicology Series: 169



LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Oral Toxicity of Nitrosoguanidine in ICR Mice (Toxicology Series [illegible]
[illegible] et al.

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This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

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for William C Cobb 11 Sept 89

Donald G. Corby (date)
COL, MC
Commanding

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# REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

| | |
|---|---|
| 1a. REPORT SECURITY CLASSIFICATION<br>UNCLASSIFIED | 1b. RESTRICTIVE MARKINGS |
| 2a. SECURITY CLASSIFICATION AUTHORITY | 3. DISTRIBUTION/AVAILABILITY OF REPORT<br>APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED. |
| 2b. DECLASSIFICATION/DOWNGRADING SCHEDULE | |
| 4. PERFORMING ORGANIZATION REPORT NUMBER(S)<br>Institute Report No.: 354 | 5. MONITORING ORGANIZATION REPORT NUMBER(S) |
| 6a. NAME OF PERFORMING ORGANIZATION<br>Mammalian Toxicology<br>Division of Toxicology<br>6b. OFFICE SYMBOL (If applicable)<br>SGRD-ULE-T | 7a. NAME OF MONITORING ORGANIZATION<br>US Army Biomedical Research and Development Laboratory |
| 6c. ADDRESS (City, State, and ZIP Code)<br>Letterman Army Institute of Research<br>Presidio of San Francisco, CA 94129-6800 | 7b. ADDRESS (City, State, and ZIP Code)<br>Fort Detrick<br>Frederick, MD 21701-5010 |
| 8a. NAME OF FUNDING/SPONSORING ORGANIZATION<br>US Army Medical Research & Development Command<br>8b. OFFICE SYMBOL (If applicable) | 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER |
| 8c. ADDRESS (City, State, and ZIP Code)<br>Fort Detrick<br>Frederick, Maryland 21701-5012 | 10. SOURCE OF FUNDING NUMBERS<br>PROGRAM ELEMENT NO.: 62720<br>PROJECT NO.: A835<br>TASK NO.: AB<br>WORK UNIT ACCESSION NO.: DA303913 |

11. TITLE (Include Security Classification)

(U) Acute Oral Toxicity of Nitrosoguanidine in ICR Mice

12. PERSONAL AUTHOR(S)

EW Morgan and DW Korte, Jr.

| 13a. TYPE OF REPORT | 13b. TIME COVERED | 14. DATE OF REPORT (Year, Month, Day) | 15. PAGE COUNT |
|---|---|---|---|
| Institute | FROM 17DEC85 TO 9JAN86 | September 1989 | 20 |

16. SUPPLEMENTARY NOTATION

Toxicology Series No. 169

| 17. COSATI CODES | | |
|---|---|---|
| FIELD | GROUP | SUB-GROUP |
| | | |
| | | |

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Acute oral toxicity; Nitrosoguanidine; Mammalian Toxicology; Mouse; Propellent; Nitroguanidine;

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The acute oral toxicity of nitrosoguanidine was determined in male and female ICR mice by using the oral gavage single-dose method. The median lethal dose was greater than a "limit dose" of 5000 mg/kg. Clinical signs observed were yellow perianal stains in 2 of 5 males and irritability in 1 of 6 females. These signs were minimal in both severity and duration. According to the classification scheme of Hodge and Sterner, these results place nitrosoguanidine in the practically nontoxic class of chemicals.

| 20. DISTRIBUTION/AVAILABILITY OF ABSTRACT | 21. ABSTRACT SECURITY CLASSIFICATION |
|---|---|
| ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS | UNCLASSIFIED |
| 22a. NAME OF RESPONSIBLE INDIVIDUAL<br>DONALD G. CORBY, COL, MC | 22b. TELEPHONE (Include Area Code)<br>(415) 561-3600<br>22c. OFFICE SYMBOL<br>SGRD-ULZ |

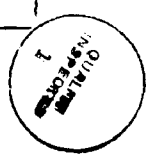
DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

## ABSTRACT

The acute oral toxicity of nitrosoguanidine was determined in male and female ICR mice by using the oral gavage single-dose method. The median lethal dose was greater than a "limit dose" of 5000 mg/kg. Clinical signs observed were yellow perianal stains in 2 of 5 males and irritability in 1 of 6 females. These signs were minimal in both severity and duration. According to the classification scheme of Hodge and Sterner, these results place nitrosoguanidine in the practically nontoxic class of chemicals.

KEY WORDS: Acute Oral Toxicity, Nitrosoguanidine, Mammalian Toxicology, Mouse, Propellant, Nitroguanidine





## PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Command
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 85009

STUDY DIRECTOR: Don W. Korte, Jr., PhD, LTC, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: Earl W. Morgan, DVM, MAJ, VC
Diplomate, American College of Veterinary Preventive Medicine, American Board of Toxicology

PATHOLOGIST: Michael V. Slayter, DVM, MAJ, VC

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, SOPs, raw data, analytical, stability, and purity data of the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Nitrosoguanidine

INCLUSIVE STUDY DATES: 17 December 1985 - 9 January 1986

OBJECTIVE: The objective of this study was to determine the acute oral toxicity of nitrosoguanidine in male and female ICR mice.

## ACKNOWLEDGMENTS

SP4 James J. Fischer, SP4 Scott L. Schwebe, and Yvonne C. LeTellier provided research assistance; SP4 Theresa L. Polk, Richard D. Spieler, and Obie Goodrich provided animal care; SGT Paul B. Simboli, BS, and SGT John R.G. Ryabik, BS, conducted chemical assays. Colleen S. Kamiyama, Ann L. Wilkinson, and Julie A. Peacock provided administrative and clerical support during the performance of this study and preparation of the report.

## SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS

We, the undersigned, declare that GLP study number 85009 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 7 Sep 89

DON W. KORTE, JR., PhD/DATE
LTC, MS
Study Director

Earl W. Morgan 24 Jun 87

EARL W. MORGAN, DVM / DATE
MAJ, VC
Principal Investigator

Conrad Wheeler 7 Sep 89

CONRAD R. WHEELER, PhD/DATE
DAC
Analytical Chemist

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8 September 1989

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SUBJECT: GLP Compliance for GLP Study 85009

1. This is to certify that the protocol for LAIR GLP Study 85009 was reviewed on 10 May 1985.

2. The institute report entitled "Acute Oral Toxicity of Nitrosoguanidine in ICR Mice," Toxicology Series 169, was audited on 6 September 1989.

Carolyn M. Lewis

CAROLYN M. LEWIS, MS
Diplomate, American Board of
Toxicology
Quality Assurance Auditor

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**Acute Oral Toxicity of Nitrosoguanidine in ICR Mice** -- Morgan *et al.*

## INTRODUCTION

Nitrosoguanidine is a potential anaerobic degradation product of nitroguanidine (1), a primary component of US Army triple-base propellants, which is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique pollutants generated by US Army munitions-manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (2). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products.

### Objective of Study

The objective of this study was to determine the acute oral toxicity of nitrosoguanidine in male and female ICR mice.

## MATERIALS

### Test Substance

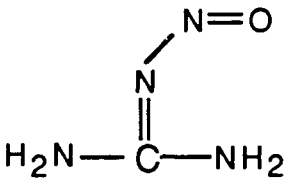
Chemical Name: Nitrosoguanidine

LAIR Code Number: TP48

Molecular Formula: $CH_4N_4O$

Physical State: Yellow powder

Chemical Structure:



Other chemical information is presented in Appendix A.

Vehicle

The vehicle for nitrosoguanidine was 1% gum tragacanth (Sigma Chemical Co., St Louis, MO), expiration March 1995, in sterile water for injection (Abbott Laboratories, North Chicago, IL), expiration 1 June 1986.

Animal Data

Seven male and 7 female ICR (CD-1) mice (Charles River Laboratories, Kingston, NY) from a shipment that arrived on 17 Dec 85 were used in this study. They were identified individually with cervical tags numbered 85C00822 to 85C00828 (males) and 85C00829 to 85C00835 (females), inclusive. Two males and two females from the shipment were selected for quality control necropsy evaluation at receipt. The animal weights on 18 Dec 85 ranged from 20 to 26 g. Additional animal data appear in Appendix B.

Husbandry

Mice were maintained individually in stainless steel wire mesh cages in racks equipped with automatically flushing dump tanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow® Diet 5002 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. The temperature of the animal room was maintained at a range from 21.7° C to 24.4° C with a relative humidity range of 38% to 60%. The photoperiod was 12 hours of light per day.

## METHODS

### Group Assignment/Acclimation

Study mice were assigned to a dose group of 7 males and 7 females. The animals were acclimated for 8 days before the day of dosing. During this period they were observed daily for signs of illness.

### Dose Levels

Since a pilot study indicated that the median lethal dose (MLD) was greater than 5000 mg/kg, a "limit dose" of 5000 mg/kg was selected for evaluating the acute oral toxicity of nitrosoguanidine.

### Preparation of Compound

Nitrosoguanidine is a yellow powder that is insoluble in water or organic liquids. It was therefore suspended at a concentration of 500 mg/ml in 1% gum tragacanth. The gum tragacanth vehicle was prepared by mixing 5 g of gum tragacanth in 495 ml of sterile water using the Kinematica model CH-6010 homogenizer.

### Chemical Analysis of Dosing Suspension

The nitrosoguanidine dosing suspension (500 mg/ml) was analyzed for accuracy and stability (Appendix A). The dosing suspension was shown to be 92.8% of the target concentration. Nitrosoguanidine suspensions have been shown to be stable (less than 2% decomposition) for 4 hours. To ensure the accuracy of the administered dose, the suspension was prepared immediately before administration. The entire procedure was completed within 30 minutes.

### Test Procedures

This study was conducted in accordance with EPA guidelines (3) and LAIR SOP-OP-STX-36 (4).

The volume of dosing suspension each animal received was based upon the desired dose level, the concentration of the compound in the suspension, and the weight of the animal. The dosing volume for all groups was based on a standard of 10 ml/kg per animal. Volumes ranged from 0.28 to 0.33 ml in the males and from 0.23 to 0.29 ml in the females. Dosing was performed using the oral gavage method without sedating the animal or administering anesthesia. Sterile disposable syringes (Becton, Dickinson & Co., Rutherford, NJ) fitted

with 18-gauge, 2-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were used for dosing. The test compound and vehicle animals were dosed between 1114 and 1130 hours on 26 December 1985.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were performed approximately 1.5, 2.5, and 5 hours after dosing, and daily for the remainder of the 2-week test period. A second "walk-through" observation was performed daily and only significant observations were recorded. Body weights were recorded once weekly during the course of the study.

Necropsy

All animals were submitted for a complete gross necropsy immediately after receiving a barbiturate overdose.

Duration of Study

Appendix C is a historical listing of study events.

Changes/Deviations

The study was accomplished according to the protocol and applicable amendments with the following exceptions:

To conserve animals, a vehicle control group was omitted. A concurrent vehicle control group from the same shipment was dosed with 1% gum tragacanth for GLP study 85016 (acute oral toxicity of JA-2 solid propellent in mice). On 31 Dec 85, the exhaust fan was inoperable for 2 hours, during this time the relative humidity rose to 78%. These deviations did not affect the outcome of this study.

Storage of Raw Data and Final Report

A copy of the final report, study protocol, raw data, retired SOPs and an aliquot of the test compound will be retained in the Letterman Army Institute of Research Archives.

## RESULTS

### Mortality

No nitrosoguanidine-induced deaths were observed. Two animals died, a male (85C00825) and a female (85C00833). At necropsy it was determined that these two mice had been misdosed. It was apparent at dosing that one other male (85C00824) was misdosed. Thus, this animal was also removed from the study.

### Clinical Observations

Eight out of eleven animals administered nitrosoguanidine appeared normal throughout the observation period. The remaining animals exhibited yellow-stained perianal regions (85C00827, 85C00828), or irritability (85C00829). All animals (except those that were misdosed) survived until termination of the study. Table 1 contains a summary of clinical observations and Appendix D contains individual animal histories.

Weight gains of survivors were not affected by dosing. Table 2 presents the mean body weights by groups. Appendix E contains individual weight tables.

### Gross Pathological Observations

No lesions were found at necropsy that could be attributed to the test compound or the test procedure. The veterinary pathologist's report appears in Appendix F.

**TABLE 1: Incidence Summary for Clinical Observations in Mice Administered a Limit Dose of Nitrosoguanidine**

**MALES**

| Category of Clinical Signs | Dose (mg/kg) (N=) | 5000 (limit) 5 |
|---|---|---|
| Stain, Yellow, Perianal | | 2 |
| Normal | | 3 |

**FEMALES**

| Category of Clinical Signs | Dose (mg/kg) (N=) | 5000 (limit) 6 |
|---|---|---|
| Irritability | | 1 |
| Normal | | 5 |

**TABLE 2: Mean Body Weights for Mice Administered a Limit Dose of Nitrosoguanidine†**

| Group | Receipt | Dosing Day | Day 7 | Day 14 |
|---|---|---|---|---|
| | | **MALES** | | |
| 5000 mg/kg | 25.0 ±0.3(7) | 30.7 ±0.7(7) | 32.4 ±0.8(5) | 33.2 ±0.8(5) |
| | | **FEMALES** | | |
| 5000 mg/kg | 20.7 ±0.3(7) | 26.1 ±0.7(7) | 28.0 ±0.8(6) | 28.7 ±0.7(6) |

† Values are the mean ± SEM (n) in g.

## DISCUSSION

The MLD for nitrosoguandine is greater than 5000 mg/kg in male and female ICR mice. This places nitrosoguanidine in the "practically non-toxic" class (5).

There was no pattern of clinical signs suggestive of a definitive clinical syndrome or a target organ system following nitrosoguanidine administration. This absence of toxicity indicates that nitrosoguanidine following oral administration has limited bioavailability since intraperitoneal doses as low as 21 mg/kg have been reported to be lethal in mice (6).

## CONCLUSION

Nitrosoguanidine is a practically nontoxic compound since it produced no significant observable effects or deaths at the "limit dose" of 5000 mg/kg in male and female ICR mice.

## REFERENCES


1. Kaplen DL, Cornell JH, Kaplen AM. Decomposition of nitroguanidine. Environ Sci Technol 1982; 16:488-492.

2. Kenyon, KF. A data base assessment of environmental fate aspects of nitroguanidine. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1982; DTIC No. ADA125591.

3. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, oral toxicity. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82- 0011.

4. Acute oral toxicity study (ALD and $LD_{50}$). LAIR Standard Operating Procedure OP-STX-36, Letterman Army Institute of Research, Presidio of San Francisco, CA. 15 June 1984.

5. Hodge HC, Sterner JH. Tabulation of toxicity classes. Amer Ind Hyg Assoc Q. 1943; 10:93-96.

6. Epstein SS, Arnold E, Andrea J, Bass W, Bishop Y. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol Appl Pharmacol 1972; 23:288-325.

## Appendix A: CHEMICAL DATA

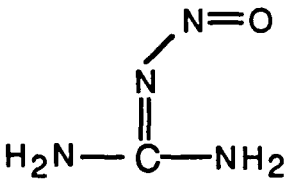
Chemical Name: Nitrosoguanidine

Chemical Abstracts Service Registry No.: 674-81-7

Lot Number: WCC-2-002

LAIR Code: TP48

Chemical Structure:



Molecular Formula: $CH_4N_4O$

Molecular Weight: 88

Physical State: Yellow powder

Analytical Data:

HPLC: Nitrosoguanidine was analyzed using conditions similar to those employed by Burrows *et al.*[1] Conditions were as follows: column, Brownlee RP-18 (4.6 mm x 25 cm); mobile phase, water; flowrate, 0.8 ml/min. The effluent was monitored at 255 nm. The retention times for nitrosoguanidine and nitroguanidine were 4.4 and 6 min, respectively. The HPLC data demonstrated that the nitrosoguanidine contained approximately 2.5% nitroguanidine.[2]

IR (KBr): 3378, 3096, 1690, 1649, 1508, 1341, 1266,1134, 1088, 1035, 690, 668 $cm^{-1}$.[3]

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[1] Burrows EP, Brueggeman EE, Hoke SH. Chromatographic trace analysis of guanidine, substituted guanidines and striazines in water. Chromatog 1984;16:494-8.

[2] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 37. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3] *Ibid.* p 30.

## Appendix A (cont.): CHEMICAL DATA

Solubility:

A saturated solution of nitrosoguanidine in water was prepared at room temperature. A 1:500 dilution of this solution produced an absorbance of 0.533 units. Using an extinction coefficient of 13,305 L/moles·cm, the concentration of nitrosoguanidine in the original saturated solution was calculated to be 1.76 mg/ml.[4]

Stability:

Stable for at least 4 hours in 1% gum tragacanth at room temperature.[5]

Source: Alan Rosencrance
US Army Biomedical Research and Development Laboratory
Fort Detrick, Maryland

Analysis of Dosing Suspension:

A suspension of nitrosoguanidine (target concentration, 500 mg/ml) was prepared using 1% gum tragacanth as the vehicle and stored overnight in a refrigerator. The next day the suspension was analyzed by UV spectroscopy as follows: An aliquot of the suspension (1 ml) was transferred to a 1000 ml volumetric flask and diluted to volume with water. A portion of this solution was diluted to 50 ml in a second volumetric flask. The absorbency of the final dilution was determined at 255 nm. The following data were used to calculate the concentration of nitrosoguanidine in the dosing suspension: 1.376 AVFS; extinction coefficient at 255 nm, 13,050; all path length, 1 cm; dilution factor, 50,000. The concentration of the dosing suspension was calculated to be 464 mg/ml or 92.8% of the target value.[6]

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[4] Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-01-006, p 66. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[5] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-12-022, p 11-13. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[6] Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 72-74. Letterman Army Institute of Research, Presidio of San Francisco, CA.

## Appendix B: ANIMAL DATA

Species: *Mus musculus*

Strain: Crl:CD-1(ICR)BR

Source: Charles River Laboratories
Kinston, NY

Sex: Male and female.

Date of birth: Male: 1 November 1985
Female: 15 October 1985

Method of randomization: First seven males and females processed from the shipment were assigned to this study.

Animals in each group: 7 male and 7 female

Condition of animals at start of study: Normal

Body weight range at dosing: 23 - 33 g

Identification procedures: Cervical tag, tag numbers from 85C00822 to 85C00835 inclusive

Pretest conditioning: Quarantine/acclimation 17 December 1985 - 25 December 1985.

Justification: The laboratory mouse has proven to be a sensitive and reliable system for lethal dose determination.

## Appendix C: HISTORICAL LISTING OF STUDY EVENTS

| Date | Event |
|---|---|
| 17 Dec 85 | Mice arrived and were checked for physical condition, sexed, and individually caged. Seven males and seven females from the shipment were assigned to the study. |
| 18 Dec 85 | All animals were weighed and cervically tagged. Two males and two females were submitted for necropsy control. |
| 18 Dec - 26 Dec 85 | Animals were observed daily. |
| 25 Dec 85 | Animals were weighed. |
| 26 Dec 85 | Food was removed from all test animals at 0730 hours. Animals were weighed and dosing was initiated at 1114 hours and concluded at 1130 hours. All animals were observed at 1.5, 2.5, and 5 hours after dosing. |
| 27 Dec 85 - 8 Jan 86 | All animals were observed daily in a.m. and p.m. |
| 2 Jan 86 | All animals were weighed. |
| 9 Jan 86 | All animals were observed, weighed, and submitted to necropsy. |

## Appendix D: INDIVIDUAL ANIMAL HISTORIES

MALES: 5000 mg/kg Nitrosoguanidine

| Animal Number | Clinical Signs | Dates Observed (1985/1986) | Severity |
|---|---|---|---|
| 84C00822 | Normal | N/A | N/A |
| 84C00823 | Normal | N/A | N/A |
| 84C00824 | Misdosed | N/A | N/A |
| 84C00825 | Misdosed | N/A | N/A |
| 84C00826 | Normal | N/A | N/A |
| 84C00827 | Stain, Yellow, Perianal | Dec 26-28 | Slight |
| 84C00828 | Stain, Yellow, Perianal | Jan 7 | Slight |

**Appendix D (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALES: 5000 mg/kg Nitrosoguanidine

| Animal Number | Clinical Signs | Dates Observed (1985/1986) | Severity |
|---|---|---|---|
| 84C00829 | Irritable | Dec 26 | Slight |
| 84C00830 | Normal | N/A | N/A |
| 84C00831 | Normal | N/A | N/A |
| 84C00832 | Normal | N/A | N/A |
| 84C00833 | Misdosed | N/A | N/A |
| 84C00834 | Normal | N/A | N/A |
| 84C00835 | Normal | N/A | N/A |

## Appendix E: INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 5000 mg/kg

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14 |
|---|---|---|---|---|
| 85C00822 | 25 | 33 | 35 | 36 |
| 85C00823 | 26 | 30 | 32 | 33 |
| 85C00824 | 25 | 31 | Misdosed | -- |
| 85C00825 | 26 | 33 | Misdosed | -- |
| 85C00826 | 25 | 30 | 32 | 33 |
| 85C00827 | 24 | 28 | 30 | 31 |
| 85C00828 | 24 | 30 | 33 | 33 |
| Mean | 25.0 | 30.7 | 32.4 | 33.2 |
| Standard Deviation | 0.8 | 1.8 | 1.8 | 1.8 |
| Std. Error of the Mean | 0.3 | 0.7 | 0.8 | 0.8 |

**Appendix E (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**

Females: 5000 mg/kg

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14 |
|---|---|---|---|---|
| 85C00829 | 21 | 29 | 31 | 31 |
| 85C00830 | 20 | 26 | 27 | 27 |
| 85C00831 | 20 | 27 | 29 | 30 |
| 85C00832 | 22 | 26 | 28 | 29 |
| 85C00833 | 21 | 26 | Misdosed | -- |
| 85C00834 | 21 | 26 | 28 | 28 |
| 85C00835 | 20 | 23 | 25 | 27 |
| Mean | 20.7 | 26.1 | 28.0 | 28.7 |
| Standard Deviation | 0.8 | 1.8 | 2.0 | 1.6 |
| Std. Error of the Mean | 0.3 | 0.7 | 0.8 | 0.7 |

# Appendix F: PATHOLOGY REPORT

Pathology Report GLP 85009
Oral $LD_{50}$, Limit Test
Investigator - CPT Morgan

Substance: Nitrosoguanidine.

Animals: Mice, ICR, Both sexes.

Reference: SOP-OP-STX-36. APC #LIB0.

Procedure:

Euthanasia - Sodium pentobarbital.
Histopathology - None.

Gross findings: The following animals were submitted live and found to have no significant gross findings.

| PATH ACC # | ANIMAL ID# |
|---|---|
| 38845 | 85C00822 |
| 38846 | 85C00823 |
| 38847 | 85C00826 |
| 38848 | 85C00827 |
| 38849 | 85C00828 |
| 38850 | 85C00829 |
| 38851 | 85C00830 |
| 38852 | 85C00831 |
| 38853 | 85C00832 |
| 38854 | 85C00834 |
| 38855 | 85C00835 |

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C, Comparative Pathology Branch

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18 February 1986

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